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Abstract

The intercalibration (IC) exercise is a key element in the implementation of the Water Framework Directive (WFD) in Europe. Its focus lies on the harmonization of national classification methods to guarantee a common understanding of 'Good Ecological Status' in surface waters. This paper defines reference conditions and sets class boundaries for deep (mean depth >15 m, IC lake type L-AL3) and moderately deep (mean depth 3–15 m, IC lake type L-AL4) Alpine lakes >0.5 km^2. Data were collated from each of the five EU member states included in the Alpine Geographical Intercalibration Group (Alpine GIG: Austria, France, Germany, Italy and Slovenia). Hydro-morphological, chemical and biological data from 161 sites (sampling stations) in 144 Alpine lakes over a period of seven decades were collated in a database. Based on a set of reference criteria, 18 L-AL3 and 13 L-AL4 reference sites were selected. Reference conditions were defined using a combined approach, based on historical, paleolimnological and monitoring data in conjunction with trophic modelling and expert judgement. Reference values and class boundaries were set for annual mean total biomass (biovolume), and then derived for annual mean chlorophyll-*a* using a regression between the two parameters. To allow for geographical differences within the Alpine GIG and to facilitate the inclusion of the broadly defined common IC types and national lake types, ranges were defined for each reference value. Range of reference values are 0.2–0.3 mg L^{-1} (L-AL3) and 0.5–0.7 mg L^{-1} (L-AL4) for total biovolume and 1.5–1.9 µg L^{-1} (L-AL3) and 2.7–3.3 µg L^{-1} (L-AL4) for chlorophyll-*a*. Depending on lake type and variable, the ecological quality ratios (EQR) for setting the class boundaries lie between 0.60 and 0.75 for the high/good class boundary and between 0.25 and 0.41 for the good/moderate class boundary. The response of sensitive phytoplankton taxa along a nutrient gradient and the occurrence of 'undesirable conditions and secondary effects' as defined in the WFD was used to validate the class boundary values, which are thus considered to be compliant with the requirements of the WFD.

Introduction

Lake assessment using phytoplankton has a long tradition in the Alpine region. It has tasked and shaped limnology from its very beginning in the late 19th century until today (Forel, 1892–1902; Vollenweider & Kerekes, 1980; Lyche Solheim et al., 2008). During the 20th century, our knowledge about the limnology of Alpine lakes steadily increased. For some lakes, there exists a continuous series of limnological data covering several decades.

Predominantly, in the second half of the 20th century, many Alpine lakes were suffering from eutrophication as an attendant symptom of increased tourism in the Alps and the growing economic prosperity. The problems arising for lakes and rivers stimulated and intensified the effort of limnological research on the functioning of aquatic ecosystems and the role of nutrients for lake productivity.

The International Biological Programme (IBP; Worthington, 1975) and the OECD research programme on eutrophication (Fricker, 1980; OECD, 1982) were two milestones for limnological research in Alpine lakes. These two programs provided a deep knowledge of the driving processes in lakes, but worked also as a platform for broad international co-operation in theoretical and applied limnology. The experience and the databases that arose from IBP and OECD programs provide the fundamental basis for this paper.

After substantial efforts to remediate Alpine lakes by various measures, such as improving the sewage treatment in the catchment area or building ring channels, the limnological monitoring during the last few decades has provided information on the re-oligotrophication process in many lakes and the response of the algal community. Data on the quantity and composition of phytoplankton is now available for Alpine lakes representative of all trophic states.

The introduction of the EU Water Framework Directive (WFD; Directive, 2000) marks a new phase of lake assessment, both from a scientific point of view (Dokulil & Teubner, 2006) and as regards the need for intensifying international co-operation and data exchange. Lake classification under the WFD is based on the degree of deviation of the present state from type-specific reference conditions.

Phytoplankton is one of the biological quality elements which are to be used for lake classification. Section 1.1.2 of Annex V of the WFD names the following criteria for lake assessment using phytoplankton: composition, abundance and biomass. This paper deals with the quantitative aspect of phytoplankton assessment in Alpine lakes larger than 0.5 km^2 and located between 50 and 800 m above sea level (a.s.l.). The objectives are to describe type-specific reference conditions and the process of class boundary setting for phytoplankton biomass and chlorophyll-*a*. The paper is the outcome of an ongoing intercalibration (IC) exercise, which is carried out in order to ensure comparability of the ecological classification scales and to obtain a common understanding of the good ecological status of surface waters throughout the EU (CIS, 2003a).

Material and methods

Lake typology

The Alpine Geographical Intercalibration Group (GIG), which comprises Austria, France, Germany, Italy and Slovenia, defined two common IC lake types. They are characterized by few and broad criteria including altitude, mean lake depth, lake surface area and alkalinity (Table 1). The values given for these descriptors are not strict boundaries, but have to be regarded as estimates, which may help assign Alpine lakes to two groups of lakes with comparable abiotic and biological reference conditions.

Sampling sites and dates

A database of 161 sites (sampling stations) in 144 Alpine lakes was compiled, which included abiotic parameters as well as data on total phosphorus, total biovolume and chlorophyll-*a*. Most of the lakes belonged to the IC common types L-AL3 and L-AL4 (Table 2). In order to broaden the data base some smaller lakes, which did not significantly differ from L-AL4 lakes in terms of hydro-morphology except for the lake area, were included in the regression analyses. The IC criteria were, however, more strictly applied to the site selection for the calculation of ranges of reference values. Very large and deep lakes (mean depth >100 m) were treated separately in the regression analyses, but not defined as separate lake type for the definition of reference values.

The number of years with data on total phytoplankton biomass ranged from 1 to 57 per site, total number of site-years with biomass data was 783 in 116 lakes, with some time series starting as early as the 1930s and ending in 2005. Total phosphorus data were available for 764 site-years in 134 lakes, chlorophyll-*a* data for 463 site-years in 126 lakes. For 275 site-years, a complete data set with TP, total biovolume and chlorophyll-*a* was available.

In most cases only one sampling site was sampled in each lake. Lake basins of some highly structured lakes are regarded as separate sites in most monitoring programs. The data from these sites were treated separately in the analyses and were not used to calculate means for the whole lake.

In most cases only years with at least four sampling dates per year were considered for the data analysis. From some sites with low interannual trophic variability, where phytoplankton data from subsequent years were available, also site-years with less than 4 sampling dates were included in the analyses.

Eight lakes with historical phytoplankton biomass data given in Ruttner (1937) were not treated as separate sites. The data were, however, averaged and treated as one data set in the analyses, in order to minimize errors due to methodological uncertainties (e.g. a lower sampling frequency). For facilitating the readability, this data set is termed and referred to as 'site' in the subsequent text.

Sampling of phytoplankton and chlorophyll-a usually occurred during whole year, including the spring peak of diatoms. All data stemmed from integrated or mixed samples from the epilimnion or the euphotic zone. No data from single depth samples were included in the database.

*Chlorophyll-*a *and biomass analysis*

Chlorophyll-*a* data were available commencing from 1972. Chl-*a* concentrations were analysed with spectrophotometry according to the procedure described in ISO (1985). Ethanol or acetone was used as extraction solvent. All chlorophyll-*a* data are turbidity corrected following Lorenzen (1967).

Total phytoplankton biomass (including picoplankton) was calculated as total biovolume following Utermöhl (1958). Abundance counts and biomass calculations were performed following the principles as outlined in EN (2006) and CEN (2007).

Definition of natural trophic state

The Alpine GIG mainly followed the spatial approach using monitoring sites in defining reference conditions (*cf* CIS, 2003b). In addition, historical data (temporally based reference conditions), modelling of anthropogenic nutrient load or natural trophic state, and expert judgement were also used:

i) Historical data: Earliest information on trophic state can be derived from data on transparency (e.g. Halbfaß, 1923). Historical quantitative data on phytoplankton in Alpine lakes are available from the 1930s for Carinthian lakes (Findenegg, 1935, 1954) and for several lakes in the Northern Calcareous Alps (Ruttner, 1937). Since these lakes were not affected by major anthropogenic pressure from industrialisation, intensive urbanisation or agriculture, the 1930s reflect reference conditions with insignificant anthropogenic impact.

In only a few Alpine lakes intensive urbanisation had led to an increased discharge of nutrients into lakes and subsequent eutrophication already in the 19th century (e.g. Amann 1918). This is confirmed by paleolimnological data that indicates that some Alpine lakes have suffered from anthropogenic eutrophication more than 100 years ago due to major urbanisation (e.g. Feuillade et al., 1995; Guilizzoni & Lami, 1992). The time before the Second World War can thus be accepted as a "reference period" only if impacts from anthropogenic land use and urbanisation were negligible.

ii) Paleolimnology: Paleolimnological data have been checked for many lakes and indicated that oligotrophic nature of many Alpine lakes (e.g. Löffler, 1978; Guilizzoni et al., 1982; Klee & Schmidt, 1987; Schmidt, 1989; Schaumburg, 1996; Klee et al., 1993; Marchetto & Bettinetti, 1995; Alefs et al., 1996; Loizeau et al., 2001; Marchetto & Musazzi, 2001). However, paleolimnology has also proved that some Alpine lakes clearly were oligo-mesotrophic or even slightly eutrophic before any significant anthropogenic impact (e.g. Frey 1955, Löffler 1978, Danielopol et al. 1985, Higgit et al. 1991, Lotter 2001, Schmidt et al. 2002, Hofmann & Schaumburg 2005). This was the case especially for some small, shallow and meromictic lakes, which naturally had reached higher levels of productivity.

iii) Modelling data: Theoretical considerations using the Vollenweider phosphorus loading model (Vollenweider, 1976; OECD, 1982) were used to check the natural trophic state of Alpine lakes. This was done by converting the critical total phosphorus load after Vollenweider (1976) at the boundary oligo-/mesotrophy

$$L_c = 10 q_s \left(1 + \sqrt{\frac{z_m}{q_s}}\right) \text{ (Eq. 1)}$$

Where L_c = critical TP load [mg m^{-2}]; $q_s = Q/A = z_m/\tau_w$ = hydraulic load [m a^{-1}]; Q = annual discharge [m^3 a^{-1}]; A = lake surface area [km^2] and Z_m = mean depth [m] to a critical export rate, ER_c:

$$ER_c = L_c \frac{A}{E} 100 \text{ (Eq. 2)}$$

For some shallow to moderately deep Alpine lakes, the potential natural TP export rate turned out to be significantly lower than the critical export rate, if the catchment is assumed to be entirely covered by forest (Fig. 1).

Definition of reference conditions using monitoring data

Two sets of criteria were used by the Alpine GIG to select reference lakes from monitoring data: i) general reference criteria, which focus on the level of anthropogenic pressure exerted on reference lakes, and ii) specific reference criteria, which focus on ecological changes caused by the anthropogenic pressure.

The criteria are based on the general requirements for the selection of reference sites following the 'Refcond Guidance' (CIS, 2003b). They describe the level of anthropogenic pressure in terms of catchment use, direct nutrient input, hydrological and morphological changes, recreation pressure etc (Table 3). These descriptors were not used as strict exclusion/inclusion criteria, especially those of minor relevance for trophic state and phytoplankton such as connectivity to tributaries or presence of non-indigenous species.

In a second step, specific reference criteria, which focussed particularly on eutrophication, were defined (Table 4). Since phosphorus is the limiting factor for primary productivity in almost all Alpine lakes and data on the TP concentration (volume weighted annual mean or during spring overturn) were available in most cases, a threshold value of the TP concentration was used for a pre-selection of reference sites. Examples from the literature show that a significant increase of phytoplankton biomass may occur already below a TP concentration of 10 µg L^{-1}, but also the taxonomic composition of planktonic algae may change along a TP gradient of 5 to 10 µg L^{-1} (e.g. Fricker, 1980; BMGU & BMWF, 1983; IGKB 2004a, b). Hence, a TP threshold value of ≤8 µg L^{-1} was defined to select reference sites among L-AL3 lakes. The slightly higher natural trophic state of shallow and moderately deep lakes was taken into account, when a threshold value of TP ≤12 µg L^{-1} was set for selecting reference sites among (pre-)Alpine lakes of IC type L-AL4.

The TP threshold values were not used for selecting reference sites in two cases: 1) Sites with slightly higher TP concentrations were also accepted as reference sites if nutrient load calculations had proved that the anthropogenic contribution to the total nutrient load was insignificant. 2) Sites that underwent a re-oligotrophication process were not considered as reference sites even if they met the TP criterion, as long as TP and chlorophyll-*a* concentrations were still declining. A delay in re-oligotrophication and especially in the response in one or more biological quality elements has been shown by Anneville & Pelletier (2000) and Kaiblinger et al. (submitted).

Setting of reference and class boundary values

For each lake, the arithmetic mean of total biomass was calculated by using data from all years available. The median of biomass values from the population of reference lakes was defined as the reference value and the 95th percentile as the high/good (H/G) boundary. The reference value and the H/G class boundary for chlorophyll-*a* were derived from a regression with phytoplankton total biomass.

The boundaries between good and moderate status were set, in a first step, by adopting boundary values suggested by Nixdorf et al. (2005) for deep Alpine lakes (L-AL3). In a second step, a 2- to 3-fold increase of phytoplankton biomass was proposed as tolerable within the good status. This is considered as being compliant with 'slight changes in the abundance' as defined in Annex V of the WFD. The values derived as such were validated by the 'undesirable conditions and secondary effects' (Annex V of the WFD) as well as by the decline of sensitive taxa, such as some *Cyclotella* species, which commonly dominate under oligotrophic conditions in Alpine lakes (e.g. Wunsam et al., 1995; Schaumburg et al., 2005). Finally, the good/moderate (G/M) boundary for phytoplankton total biomass was fixed by defining equal class widths on a logarithmic scale. The same class widths – applied to different H/G boundaries as starting points – were used for IC lake type L-AL3 and L-AL4. Like for the reference value and H/G boundary, the G/M boundary of the chlorophyll-*a* concentration was derived from a regression with phytoplankton total biomass.

For all class boundaries, ecological quality ratios (EQR) were calculated by dividing the class boundary values by the corresponding reference value.

Ranges for reference values and class boundaries of biomass and chlorophyll-a

Following an initial first test phase of lake classifications in the Alpine GIG, ranges of reference and class boundary values rather than fixed values were defined. This was in order to cover geographical or other typological differences within the Alpine region and to facilitate transposing the values of the common IC types to more detailed national typologies.

The range for the reference value of L-AL3 lakes was set using the uncertainty in the regression equation (95% confidence interval) between trophic pressure (TP concentration) and phytoplankton response (total biomass). The ranges for the class boundaries of L-AL3 lakes were derived by applying the same EQR as given for the fixed values. All values were finally rounded to one digit.

The range for the reference value of L-AL4 lakes was derived by combining two approaches: 1) by re-calculating the reference value and boundaries with new data (from 2006), but applying the same boundary setting protocol, 2) by varying the set of lakes used in the calculations (*i.e.* by excluding two pre-selected reference sites with a surface area <0.2 ha). The ranges for the class boundaries of L-AL4 lakes were subsequently set in the same way as for L-AL3, *viz*. by applying the same EQR as given for L-AL4.

Results

The range of TP concentration in the lakes used for this paper was 2 to 407 µg L^{-1}. Total biovolume data ranged from 0.1 to 10.2 mg L^{-1}, chlorophyll-*a* concentration between 0.3 and 75.8 µg L^{-1} (all values as annual means).

A regression between TP and total biovolume was calculated separately for three groups of lakes: L-AL3, very large and deep lakes (mean depth >100 m) and L-AL4 (including some lakes <50 ha) (Fig. 2). Despite the high variability (with r^2 ranging between 0.33 and 0.40), the regression coefficients and intercepts were significantly different between large lakes of L-AL3 and the other two groups (p<0.01), but not between L-AL3 and L-AL4 (p=0.33).

No significant difference between the lake types was found in the regressions of total biomass against chlorophyll-*a* (L-AL3 vs L-AL4: p>0.36, L-AL3 vs ‘L-AL3 large’: p=0.08, L-AL4 vs ‘L-AL3 large’: p=0.38). The regression equation given in Fig. 3 was thus calculated for the whole data set.

Based on the criteria given in Table 3 and 4, reference sites were selected. The historical data from the 1930s included five L-AL3 and two L-AL4 data sets (Table 5), 14 L-AL3 and 12 L-AL4 data sets came from monitoring programs after 1978 (Table 6). Since Faaker See and Weißensee, which were represented both in the historical and in the monitoring data, were treated only once, the total sum of reference sites belonging to IC lake type L-AL3 was 18, whereas 13 reference sites belonged to IC lake type L-AL4.

Biomass values of deep (L-AL3) and shallow (L-AL4) reference sites were significantly different (Mann-Whitney test, p=0.002). The median of the phytoplankton total biomass in the L-AL3 reference sites was 0.3 mg L^{-1}, the median value for 13 L-AL4 lakes was 0.7 mg L^{-1}. These values were defined as reference values for total biomass in the two lake types. The boundary between high and good ecological status, which was set at the 95% percentile, is 0.5 mg L^{-1} for L-AL3 and 1.1 mg L^{-1} for L-AL4 (Table 7).

The reference values for chlorophyll-*a*, which were calculated using the regression given in Fig. 2, are 1.9 µg L^{-1} in L-AL3 lakes and 3.3 µg L^{-1} in L-AL4 lakes. Table 8 summarizes the reference values and class boundaries for total biomass as well as for chlorophyll-*a*, which were derived according to the boundary setting protocol described above. It also gives the ecological quality ratios, which lie between 0.60 and 0.75 for the H/G boundary and between 0.25 and 0.41 for the G/M boundary.

The ranges of the reference values, which were calculated following the boundary setting protocol, are 0.2–0.3 mg L^{-1} (L-AL3) and 0.5–0.7 mg L^{-1} (L-AL4) for biomass and 1.5–1.9 µg L^{-1} (L-AL3) and 2.7–3.3 µg L^{-1} (L-AL4) for chlorophyll-a. The EQR values given in Table 8 were used to set the ranges of the class boundaries. Hence, only the absolute values are different within each lake type, whereas the EQR values are fixed. The final values for classifying lakes using phytoplankton biomass or chlorophyll-*a* are summarized in Table 9.

Discussion

The definition of type-specific reference conditions is a major prerequisite for the WFD compliant assessment of the ecological status of aquatic ecosystems. Reference conditions for phytoplankton require a clear description of the trophic state, which should have as little variability as possible within each type.

Trophic state

The trophic state as defined in this paper for deep lakes (L-AL3, mean depth >15 m) complies well with the general understanding of oligotrophy as a reference trophic state in most Alpine lakes (LAWA, 1999; Premazzi et al., 2003; Buraschi et al., 2005). The situation is less clear for moderately deep lakes belonging to GIG type L-AL4. Whereas some lakes are currently or previously oligotrophic (Hofmann & Schaumburg, 2005; KIS, 2008), historical and paleolimnological data provide evidence that others have been oligo-mesotrophic or mesotrophic prior to significant anthropogenic impact (Frey, 1955).

A WFD compliant lake classification requires, however, an assessment base, which is more precise than a general description of the trophic state. One of the key criteria used to set reference values and class boundaries for phytoplankton biomass and chlorophyll-*a* are the TP threshold values. These values are based on an extensive literature review on the relationship between nutrients and phytoplankton in Alpine lakes and supported by the modelling approach shown in Fig. 1. They are thus consistent with the normative definitions of the WFD.

The median TP values and the TP range given in Cardoso et al. (2007) for Alpine lakes also support these threshold values. They were derived independently from the Alpine GIG work using a different data set of 19 reference L-AL3 and 5 L-AL4 sites. Different to the median values, the maximum TP values for Alpine reference lakes given in Cardoso et al. (2007) appear, however, too high (L-AL3: 16 µg L^{-1}, L-AL4: 10.9 µg L^{-1}, other lake types not specified: 34.5 µg L^{-1}). These discrepancies are probably owing to the different criteria for selecting reference sites, which were less strict in Cardoso et al. (2007) than in the GIG work.

Validating the class boundaries

The class boundary between good and moderate status is doubtless the most critical one for the river basin management plans, since exceeding the G/M boundary forces the member states to set actions for improving the ecological status. A validation of the values was done using the TP – biomass response curves for several phytoplankton taxa, with sensitive *Cyclotella* species as the most important taxon. *Cyclotella* often dominates in nutrient poor Alpine lakes (Wunsam et al., 1995; Schaumburg et al., 2005) and may reach a relative proportion of annual mean total biomass of up to 95% in single years and about 2/3 for lake annual means. A decline of the relative proportion of *Cyclotella* to ≤20% corresponds to a total biomass of about 1 to 2 mg L^{-1}. The boundary set for L-AL3 lakes lies well within this range.

Another approach for validating the boundary setting is the detection of significant undesirable disturbances in the condition of other biological quality elements and the physico-chemical quality of the water or sediment. There are numerous examples from Alpine lakes in the literature, such as the decline of macrophytes, especially of charophytes and reeds (e.g. Deufel, 1978; Lachavanne, 1979; Schroeder, 1979; Melzer et al., 2003), and of white fish, *Coregonus* spp., and arctic charr, *Salvelinus umbla* (L.), with increasing eutrophication (Brutschy & Güntert, 1923; Stadelmann, 1984; Hartmann & Quoss, 1993; Gassner et al., 2003). In some lakes touristic use was heavily affected by *Planktothrix* blooms in the 1970s (Schulz et al., 2005). Nevertheless, these examples of 'undesirable conditions' can only be used for validating, but not for definitively setting the class boundaries, since the responses remain more or less descriptive in most papers and reports. The interactions between phytoplankton and other biological quality elements can vary a lot and in many cases, they are not well understood and/or quantified. Besides, they are often different in phases of eutrophication and re-oligotrophication (Dokulil et al., 2001; Anneville & Pelletier, 2000).

Weak correlations were found also for other tools for selecting reference values and setting class boundaries. Pressure criteria such as land use (using Corine land cover data) and population density equivalents were poorly correlated with trophic state, maybe because none of them include information about sewage treatment for point sources in the catchment area.

A simple site-specific predictive model based on the morpho-edaphic index MEI (Vighi & Chiaudani, 1985) was used by Cardoso et al. (2007) for defining total phosphorus concentrations under reference conditions of European lakes. According to the authors, the MEI compared favourably to more sophisticated predictive models. In the data set used for this paper, it turned out to be of limited use. Since the MEI regression of Vighi & Chiaudani (1985) estimated significantly higher TP concentrations for several (ultra-)oligotrophic lakes than are currently present, the MEI approach to defining reference status and setting boundaries was therefore considered as being not precautionary enough and was thus not pursued further.

Reference values

The reference values proposed in this paper are of the same magnitude as those published for comparable lakes in Europe by Carvalho et al. (2008) as an outcome of the REBECCA project. The chlorophyll-*a* reference value of deep Alpine lakes was 2.8 µg L^{-1} in the analysis of Carvalho et al. (2008) and thus slightly higher than those proposed for L-AL3 lakes in this paper (Table 8). This discrepancy comes mainly from the different and much smaller data set used by Carvalho et al. (2008). It included only lakes from Germany and focussed on the mean of the vegetation season, whereas the annual mean is used in this paper. In the GIG data set, the mean phytoplankton biomass in the period from March to November was 5.3% (± 1.3%, 95%C.L.) higher than the annual mean, the mean for the period April to October differed even by 9.3% (± 2.2%).

In other GIGs, reference values similar to those proposed for Alpine lakes have been found in oligotrophic northern European lakes, in contrast to the significantly higher values in moderately deep and shallow lakes in the Central-Baltic region (EU Commission, 2008). In comparable lakes in the United States, chlorophyll-*a* reference values of 1.9 µg L^{-1} (ecoregion II, Western forested mountains), 2.4 µg L^{-1} (ecoregion VIII, Nutrient poor largely glaciated upper Midwest and Northeast) and 2.8 µg L^{-1} (ecoregion XI, Central and Eastern forested uplands) were defined (U.S. EPA, 2000a,b,c). These values are broadly similar to those proposed for L-AL-3 and L-AL4, although the setting of the reference values was done in a different way (*viz.* at the 25% percentile of all values occurring in the data set).

Methodological constraints and future prospects

Apart from the boundary setting protocol, which is described in this paper, there are two aspects which strongly influence the confidence of the values: lake types, and sampling and analytical methods. Differences between sites within each of the two lake types of the Alpine GIG are probably well covered by the ranges of reference values and class boundaries as defined for L-AL3 and L-AL4.

An unknown proportion of variability, however, is owing to methodological differences in sampling (e.g., frequency, sampling depth) and analytics (e.g., counting method). The fact that there are differences in methods is not surprising facing the long period the data come from. It was tried to minimize uncertainties due to these methodological differences in the selection of the sites, which were included in the analyses.

The monitoring programs carried out in each of the Alpine countries will improve the data base by providing not only more data, but data that will bear less methodological uncertainties since they will increasingly follow international standards in sampling and analytics. Within a few years it will be possible to re-calculate reference values and class boundaries with higher confidence.

Conclusion

The protocol to define reference conditions and set boundaries as proposed by the Alpine GIG within the IC exercise and described in this paper proved to be useful and applicable. It followed a broad approach including historical, paleolimnological and monitoring data, but included also expert judgement and the experience of several decades of limnological research in Alpine lakes. The response of sensitive taxa to increased nutrient levels as well as 'undesirable conditions and secondary effects' (as described in Annex V in the WFD) was used to validate the boundaries.

The values proposed here are partly implemented in national law (e.g. Wolfram & Dokulil, 2008) and form the basis for decisions within the national river basin management plans as required by the WFD. The intercalibration between the five Alpine EU member states was thus an important step towards a harmonization of the common understanding of 'Good Ecological Status'. A second step has been made by starting the intercalibration on the taxonomic composition of phytoplankton. The final intercalibration outcome – with both biomass/chlorophyll-*a* and taxonomic composition based trophic indices included – is expected in 2010.

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Tables

Table 1. IC lake types in the Alpine GIG.

Type	Lake characterisation	Altitude [m a.s.l.]	Mean depth [m]	Alkalinity [mmol L^{-1}]	Lake size [km^2]
L-AL3	Lowland or mid-altitude, deep, moderate to high alkalinity (alpine influence), large	50–800	>15	>1	>0.5
L-AL4	Mid-altitude, shallow, moderate to high alkalinity (alpine influence), large	200–800	3–15	>1	>0.5

Table 2. Database of Alpine lakes used for the calculations. For lake types see Table 1.

	L-AL3			L-AL4			<0.5 km^2		Total		
	Lakes	Sites	Site-years	Lakes	Sites	Site-years	Lakes/Sites	Site-years	Lakes	Sites	Site-years
FR	12	14	41	5	5	5			17	19	46
IT	11	19	64	12	13	26			23	32	90
GE	16	18	138	23	23	70			39	41	208
AT	22	25	320	22	22	223	15	53	59	62	596
SI	2	2	19						2	2	19
CH	4	5	6						4	5	6
Total	67	83	588	62	63	324	15	53	144	161	965

Table 3. General reference criteria for selecting reference sites in the Alpine GIG.

Criteria	Requirement
Catchment area	>80–90% natural forest, wasteland, moors, meadows, pasture No (or insignificant) intensive crops, vines No (or insignificant) urbanisation and peri-urban areas
	No deterioration of associated wetland areas No (or insignificant) changes in the hydrological and sediment regime of the tributaries
Direct nutrient input	No direct inflow of (treated or untreated) waste water
	No (or insignificant) diffuse discharges
Hydrology	No (or insignificant) change of the natural regime (regulation, artificial rise or fall, internal circulation, withdrawal)
Morphology	No (or insignificant) artificial modifications of the shore line
Connectivity	No loss of natural connectivity for fish (upstream and downstream)
Fisheries	No introduction of fish where they were absent naturally (last decades) No fish-farming activities
Other pressures	No mass recreation (camping, swimming, rowing)
Others	No exotic or proliferating species (any plant or animal group)

Table 4. Specific criteria for selecting reference sites. The total phosphorus (TP) concentration is calculated as volume weighted annual mean or as volume weighted spring overturn concentration. Both the annual mean and the spring concentration have to remain below the suggested threshold value over at least three subsequent years.

Criteria	**Requirement**
Historical data	Prior to major industrialisation, urbanisation and intensification of agriculture
Anthropogenic nutrient load	Insignificant contribution to total nutrient load
Trophic state	No deviation of the actual from the natural trophic state Natural trophic state of L-AL3: oligotrophic (threshold value for the pre-selection of reference sites: TP ≤ 8 μg L^{-1}) Natural trophic state of L-AL4: oligo-mesotrophic (threshold value for the pre-selection of reference sites: TP ≤ 12 μg L^{-1})

Table 5. Reference sites from Alpine lakes belonging to IC lake type L-AL3 and L-AL4, based on historical data. MS = member state of the Alpine GIG (AT = Austria). BM = total phytoplankton biomass, N = number of years available for each site. Due to methodological uncertainties, the data from Ruttner (1937) are treated as one data set (site) in the analyses.

MS	Lake	IC type	Mean depth [m]	Year(s)	BM [mg L^{-1}]	N lake years
AT	Millstätter See	L-AL3	89	1932–1938	0.32	7
AT	Ossiacher See	L-AL3	20	1932–1938	0.29	7
AT	Weißensee/AT	L-AL3	37	1932–1934	0.15	21
AT	Wörthersee	L-AL3	42	1931–1938	0.29	8
AT	data from Ruttner (1937)	L-AL3	20–65	1931–1932	0.26	8
AT	Faaker See	L-AL4	16	1931–2004	0.32	5
AT	Längsee	L-AL4	13	1934–1935	0.86	2

Table 6. Reference sites from Alpine lakes belonging to IC lake type L-AL3 and L-AL4, based on the criteria in Table 3 and 4. MS = member state of the Alpine GIG (AT = Austria, FR = France, GE = Germany, IT = Italy, SI = Slovenia), *TP* = total phosphorus concentration [µg L^{-1}] (volume weighted annual mean or concentration during spring overturn). The last two columns give the number of lake years, where biovolume data were available, as well as the mean phytoplankton total biomass *BM* for these years. n.a. = no data available or site not regarded for other reasons (e.g., too few sampling dates per year).

MS	Lake	IC type	Mean depth [m]	*TP* [µg L^{-1}]	Year(s)	*BM* [mg L^{-1}]	N lake years
GE	Alpsee bei Füssen	L-AL3	28	5	2001	0.36	1
AT	Altausseer See	L-AL3	35	4	1983–2003	0.19	2
AT	Attersee	L-AL3	84	3	1989–2003	0.20	4
SI	Bohinjsko jezero	L-AL3	28	<5	1997–2005	0.15	1
AT	Fuschlsee	L-AL3	37	6	1997–2000	0.60	4
AT	Grundlsee	L-AL3	41	3–4	1981–2003	0.11	2
AT	Hallstätter See	L-AL3	65	9	2002–2003	0.06	2
GE	Königssee	L-AL3	98	5	2000	0.44	1
AT	Lunzer See	L-AL3	20	4–7	1979–1981	0.29	3
GE	Obersee/Berchtesgaden	L-AL3	30	6	2000	0.51	1
GE	Tegernsee	L-AL3	36	7	1991–1992	0.48	2
GE	Walchensee	L-AL3	81	4	1995–2003	0.31	2
AT	Weißensee/AT	L-AL3	37	5	1987–2004	0.34	18
AT	Zeller See	L-AL3	38	6	1999–2000	0.49	2
GE	Bannwaldsee	L-AL4	6	10	1997–2001	0.70	4
AT	Faaker See	L-AL4	16	6	1987–2004	0.37	18
AT	Feldsee	L-AL4	15	9	2000–2004	0.77	5
AT	Irrsee	L-AL4	15	8	2002–2003	0.59	2
AT	Keutschacher See	L-AL4	10	9	2000–2003	0.85	4
GE	Lustsee	L-AL4	6	6	1996–2000	0.35	5
AT	Magdalenensee	L-AL4	3	8	2000–2004	1.14	3
AT	Mattsee	L-AL4	17	10	1997–2000	0.29	4
AT	Pressegger See	L-AL4	3	5	2001–2004	0.22	4
AT	Rauschelesee	L-AL4	6	11	2000–2004	0.84	5
AT	Turnersee	L-AL4	8	10	2000–2003	1.03	3
GE	Wörthsee	L-AL4	15	8	1993–2002	0.43	3

Table 7. Statistics (minimum, median, arithmetic mean, percentiles) of the annual mean phytoplankton biomass [mg L^{-1}] for Alpine lakes, calculated from pre-selected reference sites (Table 5 & 6). Ref = reference value, H/G = high / good class boundary.

IC lake type	min	median Ref	mean	75% perc.	90% perc.	95% perc H/G	max	N
L-AL3	0.06	0.30	0.31	0.42	0.49	0.52	0.60	18
L-AL4	0.22	0.70	0.65	0.85	0.99	1.07	1.14	13

Table 8. Reference values, class boundaries and EQR (ecological quality ratio, in *italics*) for the annual mean total biomass [mg L^{-1}] and the annual mean chlorophyll-*a* concentration [µg L^{-1}] in Alpine lakes. H/G = class boundary high/good status, G/M = good/moderate, M/P = moderate/poor, P/B = poor/bad.

Parameter	IC lake type	Ref	H/G	G/M	M/P	P/B
Total biomass	L-AL3	0.3	0.5	1.2	3.1	7.8
[mg L^{-1}]	*EQR*	*1.00*	*0.60*	*0.25*	*0.10*	*0.04*
	L-AL4	0.7	1.1	2.7	6.9	17.4
	EQR	*1.00*	*0.64*	*0.26*	*0.10*	*0.04*
Chlorophyll-*a*	L-AL3	1.9	2.7	4.7	8.7	15.8
[µg L^{-1}]	*EQR*	*1.00*	*0.70*	*0.40*	*0.22*	*0.12*
	L-AL4	3.3	4.4	8.0	14.6	26.7
	EQR	*1.00*	*0.75*	*0.41*	*0.23*	*0.12*

Table 9. Ranges for reference values and class boundaries for the annual mean total biomass [mg L^{-1}] and the annual mean chlorophyll-*a* concentration [µg L^{-1}] in Alpine lakes. The EQR values given in Table 8 are fixed and apply to all reference values and class boundaries.

Parameter	IC lake type	Ref	H/G	G/M	M/P	P/B
Total biomass	L-AL3	0.2–0.3	0.3–0.5	0.8–1.2	2.1–3.1	5.3–7.8
[mg L^{-1}]	L-AL4	0.5–0.7	0.8–1.1	1.9–2.7	5.0–6.9	12.5–17.4
Chlorophyll-*a*	L-AL3	1.5–1.9	2.1–2.7	3.8–4.7	6.8–8.7	12.5–15.8
[µg L^{-1}]	L-AL4	2.7–3.3	3.6–4.4	6.6–8.0	11.7–14.6	22.5–26.7